PCT SEQUENCE LISTING Sequence File Name: RB125seq.txt Horwath, K. L. , Easton, C. M. , and Myers, K. <110> <120> Nucleic Acid Sequences Encoding Type III Tenebrio Antifreeze Proteins and Method for Assaying Activity. <130> RB-125-PCT <140> PCT/US01/18532 <141> 2001-06-07 <150> US 60/210,446 <151> 2000-06-08 <160> 48 <170> Microsoft Word <210> 1 <211> 19 <212> PRT <213> Tenebrio molitor <223> N-terminal sequence of protein Tm 12.86 <400> 1 Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val 19 <210> 2 <211> 576 <212> DNA <213> Tenebrio molitor <223> Non-his-tagged, signal plus, Tm 13.17 <400> 2 46 gtggatccaa agaattcggc acgagactac taag atg aag ttg ctc Met Lys Leu Leu tgt tgt cta atc tcc ctc att ctg ttg gtc aca gtt cag gcc ctg 91 Cys Cys Leu Ile Ser Leu Ile Leu Leu Val Thr Val Gln Ala Leu -10 acc gag gca caa att gag aaa ctg aac aag atc agc aaa aaa tgt 136 Thr Glu Ala Gln Ile Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys 10

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Ser Lys Lys Thr Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu 50 55 60

Val Leu Lys Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp 65 70 75 80

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				cat His												411
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	_		_		-			_			atc Ile		_		186
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	-		_					-	_		ggt Gly	_			366
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Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp 25 30 35

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			gtg gtc gac q Val Val Asp \ 69	Val Leu Arg	
			gac gaa gaa a Asp Glu Glu ! }		
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tttç	gacto	gaa t	tttt	gacaa	at aa	aaggt	acta	a tco	gttat	gta	aaaa	aaaa	aaa			645
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Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp Asp 25 30 35

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		ctg Leu														141
		caa Gln														186
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Pro Lys 40	s Met	Lys	Lys	His	Val 45	Leu	Cys	Phe	Ser	Lys 50	Arg	Thr	Gly	Val	
Ala Thi 55	Glu	Ala	Gly	Asp 60	Thr	Asn	Va1	Glu	Val 65	Leu	Lys	Ala	Lys	Leu 70	
Lys His	s Val	Ala	Ser 75	Asp	Glu	Glu	Val	Asp 80	Lys	Ile	Val	Gln	Lys 85	Cys	
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-		-		-	-		agc Ser -40		_	-	_	-				141
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gga Gly	gtg Val	gca Ala 55	acc Thr	gaa Glu	gcc Ala	gga Gly	gac Asp 60	acc Thr	aat Asn	ģtg Val	gag Glu	gta Val 65	ctc Leu	aaa Lys	3	866
acc	ааσ	cta	aad	cat	ata	acc	agg	cac	an a	a a a	ata	C a C	224	ata	,	111

Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile 70 gtg cag aag tgc gtg gtc aag aag gcc aca cca gag gaa acg gct 456 Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala tat gac acc ttc aag tgt att tac gac agt aaa cct gat ttc tct 501 Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser cct att gat taa ctcgagcacc accaccacca ccactgagat 543 Pro Ile Asp 115 <210> 39 <211> 149 <212> PRT <213> Tenebrio molitor <223> Mature protein with His-tag, Clone 7.5 Met Gly Ser Ser His His His His His Ser Ser Gly Leu Val Pro -30 Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp 105 Phe Ser Pro Ile Asp 115

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3.4, 3.9, and 7.5)
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       41
<211>
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      DNA
<212>
<213> Tenebrio molitor
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3.9, and 7.5)
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                                25
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<210> 42
<211>
      24
<212> DNA
<213> Tenebrio molitor
<223> Tm 13.17 upper primer with Bam-H1 site
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<212> DNA
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<223> Tm 13.17 lower primer with Xho1 site
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      43
gagtggtcaa ctaactgagc tcgcc
<210> 44
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<213> Tenebrio molitor
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<223> Consensus of the Tm 12.84 Isoforms, 'n' defined as any nucleotide, 'Xaa'
defined as any amino acid.
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Met Lys Leu Leu Cys Phe Ala Phe Ala Ala -15 -10

atc gtc atc gga gct cag gct ctc Ile Val Ile Gly Ala Gln Ala Leu -5 1		91							
agg aac aag atc agc aaa gar tgc Arg Asn Lys Ile Ser Lys Glu Cys 10 15		136							
caa gag acg atc gac aaa gtc cgc Gln Glu Thr Ile Asp Lys Val Arg 25 30		181							
ccc aaa atg aag aag cac gtc ctc Pro Lys Met Lys Lys His Val Leu 40 45		226							
gtg gca acc gaa gcc gga gac acc Val Ala Thr Glu Ala Gly Asp Thr 55 60		271							
aag ctg aag cat gtg gcc agc gac Lys Leu Lys His Val Ala Ser Asp 70 75		316							
cag aag tgc gtg gtc aag aag gcc Gln Lys Cys Val Val Lys Lys Ala 85 90		361							
gac acc ttc aag nnt att tac gac Asp Thr Phe Lys Xaa Ile Tyr Asp 100 105		406							
att gat taa ttgttttgta tttgrctgaa ttttgacaat aaaggtanta 455 Ile Asp 115									
tcgttatgna aaaaaaaaa aaaaaa		481							
<210> 45 <211> 484 <212> DNA <213> Tenebrio molitor									
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nng aac aag atc agc aaa rar tgy car nan gnr nny gga gtg tcn Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val Ser 10 15 20	136								
caa gag ayn atn rnc aaa gyy cgc ann ggt gnc tng gnn gay gat Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp Asp 25 30 35	181								
ccy aaa ntg aar nrn can gty yty tgc ntn ncn arg arn rcy ggn Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr Gly 40 45 50	226								
ntg gcn acn gaa ncn gga gan ryn rnn gtn gan gtr ytn arr gnn Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys Ala 55 60 65	271								
aag ntg arg nan gtn rcy rrc aac gac gaa gar ryn gan aar atc Lys Leu Lys His Val Ala Ser Asn Asp Glu Glu Val Asp Lys Ile 70 75 80	316								
rtn nan aag tgc gyn gtc aag arr gny acn nyn gar gar acg gyn Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala 85 90 95	361								
tny ray acy ttc aar nnt rty nnn ran ary aar ccn ran ttc tcn Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser 100 105 110	406								
ccn rtt gat tra nynnyynnna ytngnnnrnr nttyranaat aaagnnnntn 458 Pro Ile Asp 115									
tnrtnnnrna aaaaaaaaa aaaaaa	484								
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								acy Thr							91
								nar Gln							136
								ann Thr							181
								tgc Cys							226
								rnn Asn							271
								rnn Asp			_				316
								rnn Ala							361
								rrn Asp							406
	ryt Ile 115		trn	nynr	nnnr	nnn 3	ynngr	nnrr	ır nt	tyra	anaat	aaa	agnnr	nytn	458
tnrt	nnnı	na a	aaaa	aaaa	aa aa	aaaa	ı								484
<210 <211 <212 <213	> 4 }> [17 184 DNA Ceneb	orio	moli	ltor										
<220 <221 <222	.> n	nisc_	_feat	ure							•				
			ensus ed as					with	n AFF	P-3,	'n'	defi	.ned	as a	ny nucleotide,
<40(ggcr		17 inn a	aar a N	atg á Met I	aar y ys I	eu I	etc y eu I	mn t Leu C	gy y	rtn r he A	la F	ryy n he A	ıyy r la A	TYY Ma	46
ntn	ntn	ryc	nnr	rvv	van	acv	ntn	acv	nan	rna	nnn	ກກກ	nad	nnr	91

Ile	Val	Ile -5	Gly	Ala	Gln	Ala	Leu 1	Thr	Asp	Glu	Gln 5	Ile	Gln	Lys	
_	nny Asn 10			_						_	_		_		136
	gan Glu 25	-							-						181
_	aaa Lys 40	~						_		_	_			_	226
	ryn Ala 55		-			-	-		_	_					271
	ntn Leu 70	-									_				316
-	nnn Gln 85					_			-						361
	ran Asp 100														406
	nyn Ile 115		trn	nnnr	nnnr	nnn y	/nnri	nnnr	nn ni	nninr	nnaat	c aaa	annnr	nnn	458
nnnr	nnnr	nna a	aaaa	aaaa	aa aa	aaaa	à			•					484
<210 <211 <212 <213	l> : 2> :1	18 136 PRT Tenel	orio	moli	itor										
<220 <220 <220 <220	l> r 2>	nisc Gener	_		one 116	≈ of	Clor	165	₽1	R2 =	and 1	\ F.D_ :	۱ - ۲	n' defined	ac any
<223> General Consensus of Clones, B1, B2 and AFP-3, 'n' defined as any nucleotide, 'Xaa' defined as any amino acid															
<400 Met		18 Leu	Leu -15	Xaa	Cys	Xaa	Xaa	Xaa -10	Xaa	Xaa	Xaa	Xaa	Xaa -5	Xaa Xaa	
Xaa	Ala	Xaa 1	Thr	Xaa	Xaa	Xaa 5	Xaa	Glx	Xaa	Xaa	Xaa 10	Xaa	Xaa	Ser Xaa	•

Xaa Phe Xaa Xaa Xaa Xaa Xaa 115